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ATRAZINE TOLERANCE OF WARM-SEASON GRASS SEEDLINGS.

by

Caroline C. Bahler

A DISSERTATION

Presented to the Faculty of

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TITLE

Atrazine Tolerance of Warm-Season Grass Seedlings

BY

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ATRAZINE TOLERANCE OF WARM-SEASON GRASS SEEDLINGS

Caroline Condit Bahler, Ph.D.

University of Nebraska, 1984

Adviser: Lowell E. Moser

Weed control is a necessary component of a grass establishment program. Although atrazine [2-chloro-4-(ethyl amino)-6-(isopropylamino)-*s*-triazine] can be used with switchgrass (*Panicum virgatum* L.) and big bluestem (*Andropogon gerardii* Vitman) most warm-season grass seedlings are susceptible to atrazine. A greenhouse screening procedure was used to compare the atrazine tolerance of switchgrass seedlings with indiangrass (*Sorghastrum nutans* (L.) Nash), sideoats grama (*Bouteloua curtipendula* (Michx.) Torr.), blue grama (*Bouteloua gracilis* (H.B.K.) Lag ex Steud), 'Caucasian' bluestem (*Bothriochloa caucasica* (Trin.) C.E. Hubbard), 'Plains' bluestem (*Bothriochloa ischaemum* var *ischaemum* (L.) Keng), little bluestem (*Schizachyrium scoparium* (Michx.) Nash), and prairie sandreed (*Calamovilfa longifolia* (Hook) Schribn) seedlings. Indiangrass and sideoats grama seedlings from one cycle of field selection for atrazine tolerance were also compared. Switchgrass, Plains and Caucasian bluestem, and

prairie sandreed had the most tolerance to atrazine. Little bluestem and indiangrass (unselected and cycle 1) were intermediate, and sideoats grama (unselected and cycle 1) and blue grama had the least tolerance to atrazine.

Indiangrass and sideoats grama were used to evaluate leaf fluorescence as a possible screening technique. Concentrations of 10^{-2} or 10^{-3} M atrazine for sideoats grama or indiangrass respectively and an incubation period of 30 minutes distinquished atrazine treated leaf sections from the control. No difference was detected between established and young indiangrass and between greenhouse and field grown plants. Using a 30 minute incubation and 10^{-3} M atrazine 33 lines of young indiangrass plants were screened. Thirteen plants had a change in relative fluorescence of less than 1 which was used as the differentiation criterion. A floating leaf disk test did not differentiate among plants of differing atrazine sensitivities.

Delaying the application of atrazine 7, 14 or 26 days after planting permitted indiangrass and sideoats grama to be established in the field. In both field and greenhouse studies atrazine reduced actual seedling emergence for sideoats grama, switchgrass, and indiangrass.

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Introduction

In the temperate regions of North America there is the potential to improve summer pasture production through the use of native warm-season grasses. The practice of pasture improvement using warm-season grasses in the past has been costly in both time and money. A major problem has been that stand establishment of the warm-season grasses is slow and stand failure can occur because of weed competition. As a result many producers are reluctant to reseed even a rather poor pasture because of the expense and the risk involved.

The use of selective herbicides at seeding would reduce the risk and time needed for warm-season grass establishment. The herbicide 2,4-D [(2,4-dichlorophenoxy) acetic acid] has been used to control broadleaf weeds along with clipping. A problem with these weed control measures is that they do not control annual weedy grasses and often actually increase the annual grass competition.

Atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine] controls most troublesome annual weeds associated with warm-season seedlings during the seeding year. Established plants of native warm-season grasses are tolerant to atrazine. However, at present only switchgrass (*Panicum virgatum* L.) and big bluestem (*Andropogon gerardii* Vitman) have been proven to have sufficient atrazine

tolerance at the seedling stage to permit use of atrazine at seeding. Many other species have been proven to be susceptible to atrazine injury leading to loss of the stand at seeding time. Indiangrass (Sorghastrum nutans (L.) Nash) has been found to have marginal tolerance and sideoats grama (Bouteloua curtipendula (Michx.) Torr.) shows a high susceptibility to atrazine when the plants are in the seedling stage.

In recent years several annual weed species have developed biotypes that exhibit atrazine tolerance. These biotypes have developed in areas where atrazine has been used annually over a number of years to control vegetation in areas such as railroad right-of-ways. The discovery of resistant populations within some broadleaf weeds indicates that it may be possible to select for atrazine tolerant individuals at the seedling stage in a breeding program. A quick, inexpensive, and effective screening technique needs to be devised to facilitate atrazine resistant cultivar development.

Since there is marginal tolerance in some species the timing of the atrazine application may also affect the survival of such species. The major objectives of the following studies were to:

1.) Document the degree of atrazine tolerance in the seedling stage of some warm-season grass species;

2.) Develop screening procedures using leaf fluorescence, floating leaf sections, and plant survival in atrazine treated soil that could be used in a breeding program for improving atrazine tolerance of specific grasses;

3.) Investigate the effect of delaying atrazine application after seeding on the establishment of atrazine sensitive species; and

4.) Investigate the effect of atrazine on the emergence of warm-season grass seedlings.

Chapter One-Screening Techniques

Introduction

Atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine] is a member of the symmetrical triazine class of herbicides. The mode of action of the *s*-triazines is the inhibition of the light reactions of photosynthesis. The major use of atrazine is as a preplant incorporated, preemergence, or surface-blend herbicide, and it is used to control annual broadleaf and grassy weeds. Atrazine has also been used successfully on established native warm-season grasses as a method for controlling annual weeds in pastures. The use of atrazine would enable more effective weed control during establishment of the native warm-season grasses. The ability to quickly and reliably screen for a particular genetic trait is needed to improve a crop. The following study was designed to evaluate several screening procedures for atrazine tolerance that would be applicable to a warm-season grass breeding program.

Literature Review

Screening techniques are used to define plant responses to a given set of criteria or stresses. Screening of large numbers of plants for desirable characteristics, especially for disease resistance, was first developed in the early 1900's (Briggs and Knowles, 1967). Screening techniques can be divided into whole plant screens or screens using plant parts (leaf disks, shoots, roots, stem pieces, or plant tissues and organelles).

Whole plant screens

For some herbicides, whole plant herbicidal screens, are accomplished by incorporating known quantities of herbicide into soil. Herbicides can also be foliar applied. The species of interest is planted in the soil containing the herbicide. After a predetermined time period or growth stage plant height, fresh weight, dry weight, plant appearance, plant survival, chlorophyll content or other factors can be used to determine plant response to the herbicide (Santelmann et al, 1971; Santelmann, 1977; Winkle et al, 1981).

The major use of a whole plant screen is to test for herbicide tolerance in plant populations. Young and Evans (1978) measured etiolated growth in a field situation to determine the atrazine tolerance of crested wheatgrass

(Agropyron desertorum (Fisch. ex Link) Schult.). Van Oorschot (1965) used CO_2 uptake rates of the root to confirm the inactivation of the s-triazines and urea herbicides in whole plants. Inactivation was indicated by increased CO_2 uptake. The degree of atrazine tolerance of an individual plant or species was determined by how long it to took to reverse the decline in CO_2 uptake by the roots.

A major factor affecting the reliability of a whole plant screen where the herbicide is soil applied is the soil used. The amount of herbicide available to the plant is dependent on the adsorptive properties of the soil. Clay and organic matter content, along with the pH of the soil change the adsorptive potential of a soil. Therefore, the soil must have appropriate characteristics which permit optimum availability of the herbicide to the plant (Lavy, 1968; Green, 1974; Weed and Weber, 1974). Thorough incorporation of the herbicide into the soil is also critical. The uniformity of herbicide distribution throughout the soil and the changes in the soil's adsorptive ability through air drying, can also alter the availability and the reliability of the screen (Santelmann, 1977).

Mode of action of atrazine

Atrazine is a member of the *s*-triazine class of herbicides. This group of herbicides inhibits the light reactions of photosynthesis in photosystem II. This is accomplished by the herbicide blocking the electron flow from Q to the plastoquinone pool, B (Pfister and Arntzen, 1979; Ashton and Craft, 1981). This causes a backup of electrons at Q. Some of the excess energy is given off as heat and some fluoresced as light. Fluorescence naturally occurs at Q (which stands for fluorescence quencher) since the molecule can only pass one electron on to B. Under optimal photosynthetic conditions Q becomes saturated with electrons and fluorescence occurs because the excess energy still contained in the chlorophyll is released as photons (Rabinowitch and Govindjee, 1969; Papageorgiou, 1975).

Atrazine also causes the backup of electrons at Q by binding to a protein associated with the Q-B complex (known as a proteinaceous shield) in the thylakoid membrane. This proteinaceous shield is thought to somehow mediate the flow of electrons from Q to B. When a herbicide (*s*-triazine or urea herbicides) binds to the proteinaceous shield a change in the configuration of the protein occurs prohibiting electron flow (Pfister and Arntzen, 1979; Ashton and Craft, 1981). The amount of fluorescence increases as atrazine binding increases allowing for easy detection of fluorescence (Bohme et al, 1981).

Susceptibility of a plant to atrazine injury has been related to three factors (Shimabukuro and Swanson, 1969): 1.) the rate of atrazine metabolism and the nature of the atrazine derivatives that are formed; 2.) the concentration of atrazine in the chloroplast; and 3.) the amount of time needed for recovery of photochemical activity in the chloroplast after atrazine removal. Shimabukuro and Swanson (1969) also proposed a possible mechanism of atrazine tolerance. Atrazine accumulates in the chloroplast and cytoplasm of a mesophyll cell. As it is metabolized in the cytoplasm the amount of atrazine decreases. This causes some of the unaltered atrazine in the chloroplast to diffuse into the cytoplasm reducing the amount of atrazine in the chloroplast (Shimabukuro and Swanson, 1969).

Metabolism of atrazine can occur through three different pathways. The atrazine can be N-dealkylated, 2-hydroxylated, or form a conjugate with glutathione (Shimabukuro, 1967; Lamoureux et al, 1973). Both the peptide conjugate and the hydroxyatrazine are considered to be the complete detoxification products of atrazine having no residual phytotoxicity. However, the N-dealkylation reaction does not completely detoxify the atrazine molecule; instead a molecule that has a reduced phytotoxic ability is produced (Shimabukuro, 1967; Lamoureux et al, 1973).